January 28, 2003. The failure of the cited prior art to teach or suggest all elements of the claims, as set forth in sections D and E below, was discussed. The examiner did not comment on the substance of the rejection, but indicated that she would look over the matter. No agreement was reached.

B. Status of the Claims

Claims 1-32 were pending at the time of the second Office Action. No claim amendments have been made herein. Claims 1-32 are currently pending and presented herein for reconsideration.

C. Status of the Specification

The figure legends have been amended to conform with the formal drawings being submitted concurrently herewith. A marked copy of the amendments is attached herewith as Appendix A.

D. Drawings

The Action maintains the objection to the drawings under 37 C.F.R. § 1.84 or § 1.152. In response, Applicants note that formal drawings are being submitted herewith. It is believed that the objection is now moot in light of the submission.

E. Rejections Under 35 U.S.C. §102(b)

The Action maintains the rejection of claims 1-25 and 27-32 under 35 U.S.C. §102(b) as being anticipated by Georgiou *et al.* (U.S. Patent No. 5,866,344). Applicants respectfully traverse as set forth below.

The cited reference does not teach the claimed method. The rejection has apparently been maintained as a result of confusion regarding the distinction between cell surface expression and periplasmic expression. For example, the Action states the following on page 3:

> Georgiou teaches that a particular advantage of *cell surface (periplasm)* expressed antigen-binding antibodies is that the antibody is attached to the *outer membrane* of the cell (column 6, lines 63-65) and that the *surface* displayed antibodies or antibody conjugates may be catalytic antibodies conjugates... (emphasis added)

On page 5 and page 6 of the Action, it is stated that Georgiou *et al.* teaches candidate antibodies "that have been expressed on the surface of a host cell"; or that Georgiou teaches an assay involving a known amount of "antibody-covered cells" and that "immunocomplexes are bound to the bacterium since the detection occurs at the cell surface of the bacterium." However, each of these passages indicate the distinctness of Georgiou and fail to address the instantly claimed invention.

The allegations in the Action refer to identification of *cell surface*-bound ligands, but not "based on the presence of said labeled ligand within the bacterium" or wherein "said ligand and said candidate binding protein are bound in said bacterium." The cell surface is not the periplasm. The periplasm is within the bacterial outer wall. This is set forth on page 13 of the specification, where it is stated that the periplasm comprises the space defined by the inner and

outer membranes of a gram-negative bacterium. The Office may not properly ignore the art-known meaning of this term and definition of the term in the specification.

It is also noted that, because the antigen-binding antibodies of Georgiou *et al.* are expressed on the outer cell surface, which is specifically acknowledged in the Action, they must be ***anchored*** to the surface. That is, they are expressed in ***insoluble*** form. In contrast, step (a) of the instantly claimed invention recites:

> (a) providing a Gram negative bacterium comprising a nucleic acid sequence encoding a candidate binding protein, wherein said binding protein is expressed ***in soluble form in said bacterium***. (emphasis added)

Therefore, neither the reference nor the Action has done anything to address "wherein said binding protein is expressed in soluble form in said bacterium." The Action nonetheless takes the position that use of FACS by Georgiou *et al.* to identify antibodies could lead one to "reasonably conclude that Georgiou teaches expression of a binding protein in soluble form in a bacterium because FACS is used as a method of identifying the antibodies in the invention and FACS requires the use of soluble haptens." However, this is incorrect. Whether FACS is or is not used is irrelevant to whether a binding protein is expressed in soluble form in a bacterium. FACS is a method that allows sorting of cells exhibiting fluorescence. Such fluorescence can be achieved in numerous ways, including using a fluorescent dye. In Georgiou *et al.*, this was done by binding with fluorescently labeled compounds ***at the cell surface***. There is no basis to support the contention that this has anything to do with soluble expression.

No basis has further been provided by the Action to indicate what use of soluble haptens has to do with the invention. As indicated above, step (a) requires that a binding protein be "expressed in soluble form in said bacterium." Use of soluble haptens is distinct from expression of a binding protein in soluble form in a bacterium. Again, the Action has failed to address this

element. No basis has been provided, legal or scientific, to suggest why this could reasonably lead one to conclude that expression of a binding protein in soluble form is taught by the reference.

In view of the foregoing, Applicants respectfully request the removal of the rejection under 35 U.S.C. § 102(b).

F. Rejections Under 35 U.S.C. §103(a)

The Action rejects claims 1-32 under 35 U.S.C. §103 as allegedly being obvious over Georgiou *et al.* (U.S. Patent No. 5,866,344) in view of Pini *et al.* (*J. Biol. Chem.*, 1998, Vol. 283, No. 34, p. 21769-21776). Applicants respectfully traverse.

Pini *et al.* does nothing to remedy the shortcomings of Georgiou *et al.*, as set forth above. Pini *et al.* is alleged in the Action to teach use of phage in constructing antibody libraries and certain advantages of phage antibody display. However, it is not alleged that Pini *et al.* remedies any of the deficiencies described herein above with respect to claim 1. In particular, the Action has not addressed the element of a Gram negative bacterium comprising a nucleic acid sequence encoding a candidate binding protein, wherein the binding protein is expressed in soluble form in the bacterium, let alone the remaining steps of the invention. The closest the Action comes to this is to state on the bottom of page 7 that cell surface expression is the same as periplasmic expression. However, Applicants again note that the cell surface and periplasm are not the same, as described above.

With regard to the statement in the Action, on page 8, that Georgiou teaches cells with an antibody "displayed on the surface," Applicants direct the Examiner to step (c) of claim 1, which recites:

(c) selecting said bacterium based on the presence of said labeled ligand ***within the bacterium***, wherein said ligand and said candidate binding protein are bound ***in said bacterium*** (emphasis added)

Claim 1 therefore involves selecting the bacterium based on the presence of the labeled ligand ***within the bacterium***. The Action, however, has only addressed expression on the surface of the bacterium. Nothing has been done to address this element. In this regard, Applicants note that it is specifically the duty of the Office to show that the prior art teaches or suggests all claim limitations, as well as to provide a motivation or suggestion to combine the references to arrive at the invention. *See In re Vaeck*, 947 F.2d 488, 20 USPQ 2d 1438 (Fed. Cir. 1991), *see also*, M.P.E.P. § 2142. This burden has not been met.

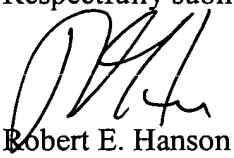
Applicants finally note that it is the burden of the Office to state with clarity the basis of the rejection. *In Re Lee*, 277 F.3d 1338, 1344-45 (Fed. Cir. 2002). In the instant case, there has been no showing that all elements of the invention have been taught, let alone suggested in combination. Nonetheless, the rejection concludes that "it would be expected barring evidence to the contrary" that the invention is taught by the prior art. It therefore appears that common knowledge, or a "gist of the invention" type approach is being used to support the rejection. This approach is expressly contrary to Federal Circuit caselaw. *Id.* Therefore, if the rejection is maintained, Applicants respectfully request that an affidavit be provided pursuant to 37 C.F.R. §1.104(d) setting forth the basis for the instant rejection with regard to all claim elements and any common knowledge relied upon therein.

In view of the foregoing, Applicants respectfully request removal of the rejection of claims 1-32 under 35 U.S.C. §103.

G. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. The examiner is invited to contact the undersigned (512)536-3085 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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Date: February 24, 2003

APPENDIX A: VERSION OF AMENDMENTS MARKED TO SHOW CHANGES

In the specification:

Please amend the paragraphs beginning on page 11, line 6, and ending on page 13, line 9:

--**FIG. 1.** General scheme of the basis of a preferred embodiment of the invention. A library of proteins expressed in the periplasmic space of bacteria is contacted with a fluorescent reagent. Bacterial clones expressing a protein having a desired activity (*e.g.*, either binding of the probe or enzymatic conversion to a product) become fluorescently labeled. The fluorescent cells can subsequently be isolated by FACS.

FIGs. 2A-2C. Isolation of affinity improved mutants of an anti-digoxin antibody by two rounds of sorting. A library of scFv mutants in which three residues in the light chain had been randomized was constructed as described in Example 2. A total of $2.5x10^6$ transformants were grown in liquid media, labeled with 100 nM digoxin-BODIPY™ and fluorescent cells falling within the window shown in the rightmost panel were sorted by FACS. The sorted cells were grown in liquid media, re-labeled and cells falling within the specified window as shown in the center panel were isolated. Following a final round of re-growth the cells were analyzed by FACS (FIG. 2A[left-most panel]). Single scFv antibody colonies were picked at random, analyzed and the affinity of the corresponding scFv proteins are reported in Table 1.

FIGs. 3A-3H. Shows strain dependence of periplasmic FACS signal: [i)]FIG. 3A TG1/pHEN2.thy; [ii)]FIG. 3C HB2151/pHEN2.thy; [iii)]FIG. 3E ABLE™C/pHEN2.thy; [iv)]FIG. 3G ABLE™K/pHEN2.thy; [v)]FIG. 3B TG1/pHEN2.dig; [vi)]FIG. 3D HB2151/pHEN2.dig; [vii)]FIG. 3F ABLE™C/pHEN2.dig; [viii)]FIG. 3H ABLE™K/pHEN2.dig.

FIGs. 4A-4H. Effect of hyperosmotic shock on labeling efficiency: [(i)–(iv),]FIG 4A, FIG. 4C, FIG. 4E, FIG. 4G: pHEN2.thy; [(v)–(viii),] FIG 4B, FIG. 4D, FIG. 4F, FIG. 4H pHEN2.dig; [(i)] FIG. 4A and [(v)] FIG. 4B, 1xPBS; [(ii)]FIG. 4C and [(vi)] FIG. 4D, 2.5xPBS; [(iii)]FIG. 4E and [(vii)]FIG. 4F 5xPBS; [(iv)]FIG. 4G and [(viii)]FIG. 4H 10xPBS.

FIGs. 5A-5D. Maximizing periplasmic FACS signal in ABLE™C labeled in 5xPBS using P_{tac} vector and superinfection with M13KO7 (moi of 10) 0.5h pre-induction: [i)]FIG. 5A pHEN.thy; [ii)]FIG. 5C pHEN2.thy/M13K07; pHEN2.dig; [iv)] FIG. 5D pHEN2.dig/M13K07.

FIGs. 6A-6C. FIG 6A: Phage eluate titers, after each round of panning. FIG. 6B: Polyclonal phage ELISA of purified phage stocks on digoxin-ovalbumin. FIG. 6C: FACScanning naïve library FIG. 6C-1[(i)] and rounds one to five ([ii]FIG. 6C-2 to [vi]FIG. 6C-6) of panning on digoxin-BSA using BODIPY™-digoxygenin.

FIGs. 7A, 7B. Amino acid and nucleotide sequences of scFv antibody fragments isolated by expression in the periplasm and FACS. FIG. 7A: Heavy chain of dig1 is shown in true font while dig3 is shown in italics underneath. The nucleotide sequences corresponding to the heavy chains of dig1 and dig 3 are given by SEQ ID NO:17 and SEQ ID NO:18, respectively. Dig2

variation from dig 1 is as indicated in underlined text within CDR3. FIG. 7B: Light chain of digl, 2 and 3 with variations in CDR3 indicated as for heavy chain. The nucleotide sequences corresponding to the light chains of digl and dig 3 are given by SEQ ID NO:19 and SEQ ID NO:20, respectively. The underlined four nucleotide variation beginning at nucleotide 99 is given by SEQ ID NO: 21.

FIGs. 8A-8D. Labeling of periplasmic scFv by fluorescently tagged oligonucleotide probe. ABLE^{TM}C cells expressing periplasmic scFv specific for either atrazine as a negative control ([i]FIG. 8A and [iii]FIG. 8C) or for digoxin ([ii]FIG. 8B and [iv]FIG. 8D) were labeled either with: 100nM with digoxigenin-BODIPY™ ([i]FIG. 8A and [ii]FIG. 8B) or 100nM of dig-5A-FL ([iii]FIG. 8C and [iv]FIG. 8D). 10,000 events were recorded using a FACSort flow cytometer at a rate of approximately 1,000 events per second.

FIGs. 9A-9B. Fluorescence discrimination of *E. coli* expressing the enzyme cutinase (an esterase) from control bacteria not expressing the enzyme. *E. coli* DH5a cells were transformed either with the plasmid pBAD18Cm (control cells) or with the derivative plasmid pKG3-53-1 encoding the *Fusarium solani* enzyme cutinase. FIG. 9A. Fluorescence histogram showing the selective labeling of *E. coli* expressing cutinase in the periplasm (pkg3-53-1 containing-cells) using a fluorescent esterase substrate (10μM Fluorescein Dibutyrate) for 30 minutes at 37°C. FIG. 9B. Fluorescence histogram of from selective labeling of cutinase-expressing cells (transcribed from the pKG3-53-4 vector) labeled with a fluorescent pH-Sensitive Dye (1μM LysoSensor Green DND-189) in the presence of cutinase substrate (1mM 4-Nitrophenyl Butyrate). The cells were labeled for 5 minutes at 25°C. Acidification of the periplasm occurred as a result of ester hydrolysis by the cutinase.--